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(54) Title: TREATMENT OF TYPE II DIABETES MELLITUS WITH AMYLIN AGONISTS

(57) Abstract

Methods for treating non-insulin-taking Type II diabetes mellituswhich comprise administering a therapeutically effective amount of an amylin agonist.

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DESCRIPTION

Treatment of Type II Diabetes Mellitus
With Amylin Agonists

Field of the Invention

The present invention relates to medicine, and,
5 more particularly, to amylin agonists and methods for the
treatment of Type II diabetes mellitus by administering an
amylin agonist.

Background of the Invention

Publications and other materials including
10 patents and patent applications used to illuminate the
specification are hereby incorporated by reference.

Amylin

Amylin is a 37 amino acid protein hormone. It
was isolated, purified and chemically characterized as the
15 major component of amyloid deposits in the islets of
pancreases of human Type II diabetics (Cooper et al.,
Proc. Natl. Acad. Sci., USA 84:8628-8632 (1987)). The
amylin molecule has two important post-translational
modifications: the C-terminus is amidated, and the cystei-
20 nes in positions 2 and 7 are cross-linked to form an
N-terminal loop. Amylin is the subject of United Kingdom
patent application Serial No. 8709871, filed April 27,
1987, and corresponding United States Patent No. 5,367,05-
2, issued November 22, 1994.

Amylin is a member of a family of related pepti-
des which include CGRP and calcitonin (Rink et al., Trends
Pharmacol. Sci. 14:113-118 (1993)). Amylin is primarily
synthesized in pancreatic beta cells and is secreted in
response to nutrient stimuli such as glucose and arginine.
30 Moore et al., Biochem. Biophys. Res. Commun. 179:1-9
(1991); Kanatsuka et al., FEBS Lett. 259:199-201 (1989);
Ogawa et al., J. Clin. Invest. 85:973-976 (1990); Gedulin

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et al., Biochem. Biophys. Res. Commun. 180:782-789 (1991).

In normal humans, fasting amylin levels from 1 to 10 pM and post-prandial levels of 5 to 20pM have been reported (e.g., Hartter et al., Diabetologia 34:52-54 5 (1991); Sanke et al., Diabetologia 34:129-132 (1991)); Koda et al., The Lancet 339:1179-1180 (1992)). In obese, insulin-resistant individuals, however, post-food amylin levels can go higher, reaching up to about 50 pM, for example. For comparison, the values for fasting and post- 10 prandial insulin are 20 to 50 pM, and 100 to 300 pM respectively in healthy people, with perhaps 3-to 4-fold higher levels in insulin-resistant people. In Type 1 diabetes, where beta-cells are destroyed, amylin levels are at or below the levels of detection and do not rise in 15 response to glucose (Koda et al., The Lancet 339:1179-1180 (1992)). In normal mice and rats, basal amylin levels have been reported from 30 to 100 pM, while values up to 600 pM have been measured in certain insulin-resistant, diabetic strains of rodents (e.g., Huang et al., Hyperten- 20 sion 19:I-101-I-109 (1991)); Gill et al., Life Sciences 48:703-710 (1991).

The first discovered action of amylin was the reduction of insulin-stimulated incorporation of glucose into glycogen in rat skeletal muscle (Leighton et al., 25 Nature 335:632-635 (1988)); the muscle was made "insulin-resistant". Subsequent work with rat soleus muscle ex-vivo and in vitro has indicated that amylin reduces glycogen-synthase activity, promotes conversion of glyco-gen phosphorylase from the inactive b form to the active 30 a form, promotes net loss of glycogen (in the presence or absence of insulin), increases glucose-6-phosphate levels, and can increase lactate output (see, e.g., Deems et al., Biochem. Biophys. Res. Commun. 181:116-120 (1991)); Young et al., FEBS Letts. 281:149-151 (1991)).

35   It is believed that amylin acts through receptors present in plasma membranes. Beaumont et al., Mol. Pharmacol. 44:493-497 (1993). Amylin receptors and their

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use in various methods for screening and assaying for amylin agonist and antagonist compounds are described in United States Patent No. 5,264,372, issued November 23, 1993.

5   The biological actions of amylin relating to fuel metabolism are discussed in Young et al., J. Cell. Biochem. 555:12-18 (1994). While amylin has marked effects on hepatic fuel metabolism in vivo, there is no general agreement as to what amylin actions are seen in isolated

10   hepatocytes or perfused liver. The available data do not support the idea that amylin promotes hepatic glycogenolysis, i.e., it does not act like glucagon (e.g., Stephens, et al., Diabetes 40:395-400 (1991)); Gomez-Foix et al., Biochem J. 276:607-610 (1991)). It has been suggested

15   that amylin may act on the liver to promote conversion of lactate to glycogen and to enhance the amount of glucose able to be liberated by glucagon (see Roden et al., Diabetologia 35:116-120 (1992)). Thus, amylin could act there as an anabolic partner to insulin in liver, in

20   contrast to its catabolic action in muscle.

In fat cells, contrary to its action in muscle, amylin has no detectable actions on insulin-stimulated glucose uptake, incorporation of glucose into triglyceride, CO_2 production (Cooper et al., Proc. Natl. Acad. Sci.

25   85:7763-7766 (1988)) epinephrine-stimulated lipolysis, or insulin-inhibition of lipolysis (Lupien and Young, Diabetes Nutrition and Metabolism - Clinical and Experimental, Vol. 6(1), pages 13-18 (1993)). Amylin thus exerts tissue-specific effects, with direct action on skeletal

30   muscle, marked indirect (via supply of substrate) and perhaps direct effects on liver, while adipocytes appear "blind" to the presence or absence of amylin. No direct effects of amylin on kidney tissue have been reported.

It has also been reported that amylin can have

35   marked effects on secretion of insulin. In isolated islets (Ohsawa et al., Biochem. Biophys. Res. Commun. 160:961-967 (1989)), in the perfused pancreas (Silvestre

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et al., Reg. Pept. 31:23-31 (1991)), and in the intact rat
(Young et al., Mol. Cell. Endocrinol. 84:R1-R5 (1992)),
various experiments indicate that amylin down-regulates
insulin secretion. The perfused pancreas experiments
5   point to selective down-regulation of the secretary
response to glucose with sparing of the response to
arginine. Other workers, however, have been unable to
detect effects of amylin on isolated beta cells, on
isolated islets, or in the whole animal (see Broderick et
10 al., Biochem. Biophys. Res. Commun. 177:932-938 (1991) and
references therein).

A striking effect of amylin in rodents at pharma-
cological dosages in vivo is stimulation of a sharp rise
in plasma lactate, followed by a rise in plasma glucose
15 (Young et al., FEBS Letts. 281:149-151 (1991)). Evidence
indicates that the increased lactate provides substrate
for glucose production and that amylin actions can occur
independent of changes in insulin or glucagon. In "glu-
cose clamp" experiments, amylin infusions cause "insulin
20 resistance", both by reducing peripheral glucose disposal,
and by limiting insulin-mediated suppression of hepatic
glucose output (e.g., Frontoni et al., Diabetes 40:568-573
(1991); Koopmans et al., Diabetologia 34:218-224 (1991)).

It has been shown that amylin agonists can slow
25 gastric emptying (Young et al., Diabetologia (June 1995,
in press), and is believed to contribute to their ability
to reduce post-prandial hyperglycemia (Moyses and Kolterm-
an, Drugs of the Future (May 1995). Methods for reducing
gastric motility and slowing gastric emptying comprising
30 the administration of an amylin agonist (including amylin)
are the subject of United States Patent Application Serial
No. 08/118,381, filed September 7, 1993, and United States
Patent Application Serial No. 08/302,069, filed September
7, 1994 (and corresponding PCT application, Publication
35 No. WO 95/07098, published March 16, 1995).

Non-metabolic actions of amylin include vasodila-
tor effects which may be mediated by interaction with CGRP

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vascular receptors. (Brain et al., Eur. J. Pharmacol. 183:2221 (1990). It has also been reported that amylin markedly increases plasma renin activity in intact rats when given subcutaneously in a manner that avoids any

5   disturbance of blood pressure. Methods for treating renin-related disorders with amylin antagonists are described in United States Patent No. 5,376,638, issued December 27, 1994.

        Injected into the brain, amylin has been reported

10 to suppress food intake (e.g., Chance et al., Brain Res. 539:352-354 (1991)), an action shared with CGRP and calcitonin. The effective concentrations at the cells that mediate this action are not known. Amylin has also been reported to have effects both on isolated osteoclasts

15 where it caused cell quiescence, and in vivo where it was reported to lower plasma calcium by up to 20% in rats, in rabbits, and in humans with Paget's disease (see, e.g., Zaidi et al., J. Bone Mineral Res. 5(Suppl. 2) 576 (1990). From the available data, amylin seems to be 10 to 30 times

20 less potent than human calcitonin for these actions. Interestingly, it was reported that amylin appeared to increase osteoclast cAMP production but not to increase cytosolic Ca^{2+}, while calcitonin does both (Alam et al., Biochem. Biophys. Res. Commun. 179:134-139 (1991)). It

25 was suggested, though not established, that calcitonin may act via two receptor types and that amylin may interact with only one of these.

Diabetes Mellitus

        Diabetes mellitus is a serious metabolic disease

30 that is defined by the presence of chronically elevated levels of blood glucose. Classic symptoms of diabetes mellitus in adults are polyuria, polydipsia, ketonuria, rapid weight loss together with elevated levels of plasma glucose. Normal fasting plasma glucose concentrations are

35 less than 115 milligrams per deciliter. In diabetic patients, fasting concentrations are found to be over 140

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milligrams per deciliter. In general, diabetes mellitus develops in response to damage to the beta cells of the pancreas. This damage can result from primary diabetes mellitus, in which the beta cells are destroyed by the
5   autoimmune system, or as a secondary diabetic response to other primary diseases, such as pancreatic disease, hormonal abnormalities other than lack of insulin action, drug or chemical induction, insulin receptor abnormalities, genetic syndromes or others. Primary diabetes
10 mellitus can be classified as Type I diabetes (also called insulin-dependent diabetes ellitus or IDDM) and Type II diabetes mellitus (also called non-insulin dependent diabetes mellitus or NIDDM).

Type I (juvenile onset or insulin-dependent)
15 diabetes is a well-known hormone deficient state, in which the pancreatic beta cells appear to have been destroyed by the body's own immune defense mechanisms. Patients with Type I diabetes mellitus have little or no endogenous insulin secretory capacity. These patients develop
20 extreme hyperglycemia. Type I diabetes was fatal until the introduction of insulin replacement therapy some 70 years ago -- first using insulins from animal sources, and more recently, using human insulin made by recombinant DNA technology. As discussed above, it is now clear that the
25 destruction of beta cells in Type I diabetes leads to a combined deficiency of two hormones, insulin and amylin. When pancreatic cells are destroyed, the capacity to secrete insulin and amylin is lost. Type I diabetic subjects have reported amylin levels that are either
30 undetectable or at the lower limit of detection, and which fail to increase in response to a glucose challenge. Koda, The Lancet 339:1179 (1992).

The nature of the lesion of the pancreatic beta cells in Type II diabetes is not clear. Unlike the
35 pancreatic beta cells in Type I diabetics, the beta cells of Type II diabetics retain the ability to synthesize and secrete insulin and amylin.

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Type II diabetes is characterized by insulin and resistance, i.e., a failure of the normal metabolic response of peripheral tissues to the action of insulin. In other words, insulin resistance is a condition where

5 the circulating insulin produces a subnormal biological response. In clinical terms, insulin resistance is present when normal or elevated blood glucose levels persist in the face of normal or elevated levels of insulin. The hyperglycemia associated with Type II

10 diabetes can sometimes be reversed or ameliorated by diet or weight loss sufficient to restore the sensitivity of the peripheral tissues to insulin. Indeed, Type II diabetes mellitus is often characterized by hyperglycemia in the presence of higher than normal levels of plasma

15 insulin. Progression of Type II diabetes mellitus is associated with increasing concentrations of blood glucose and coupled with a relative decrease in the rate of glucose-induced insulin secretion. Thus, for example, in late-stage Type II diabetes mellitus, there may be an

20 insulin deficiency.

The primary aim of treatment in all forms of diabetes mellitus is the same, namely the reduction of blood glucose concentrations to as near normal as possible, thereby minimizing both the short- and long-term

25 complications of the disease. Tchobroutsky, _Diabetologia_ 15:143-152 (1978). The linkage between the extent of hyperglycemia in diabetics and the ensuing long-term complications was further confirmed in the recently completed Diabetes Control and Complications Trial (DCCT)

30 undertaken by the National Institutes of Health. The Diabetes Control and Complications Trial Research Group, _N. Eng. J. Med._ 329:977 (1993). The DCCT was conducted over a 10-year period at 29 clinical centers around the United States and Canada, and showed that lowering mean

35 blood glucose concentrations in Type I diabetics reduced end-organ complications. The development of retinopathy was reduced by 76%, the progression of retinopathy by 54%,

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and there was an amelioration of the markers of renal
disease (proteinuria, albuminuria). The development of
significant neuropathic changes was also reduced.

5   The treatment of Type I diabetes necessarily
involves the administration of replacement doses of
insulin, administered by the parenteral route. In combi-
nation with the correct diet and self-blood glucose
monitoring, the majority of Type I diabetics can achieve
a certain level of control of blood glucose.   ·

10   In contrast to Type I diabetes, treatment of Type
II diabetes frequently does not require the use of insu-
lin.   Institution of therapy in Type II diabetes
usually involves a trial of dietary therapy and lifestyle
modification, typically for 6-12 weeks in the first

15 instance. Features of a diabetic diet include an adequate
but not excessive total calorie intake, with regular
meals, restriction of the content of saturated fat, a
concomitant increase in the polyunsaturated fatty acid
content, and an increased intake of dietary fiber.

20 Lifestyle modifications include the maintenance of regular
exercise, as an aid both to weight control and also to
reduce the degree of insulin resistance. If after an
adequate trial of diet and lifestyle modifications,
fasting hyperglycemia persists, then a diagnosis of

25 "primary diet failure" may be made, and either a trial of
oral hypoglycemic therapy or direct institution of insulin
therapy will be required to produce blood glucose control
and, thereby, to minimize the complications of the dis-
ease. Type II diabetics who fail to respond to diet and

30 weight loss may respond to therapy with oral hypoglycemic
agents such as sulfonylureas or biguanides.   Insulin
therapy, however, is used to treat other patients with
Type II diabetes, especially those who have undergone
primary dietary failure and are not obese, or those who

35 have undergone both primary diet failure and secondary
oral hypoglycemic failure.

           The use of amylin agonists in the treatment of

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diabetes mellitus has been described in United States
Patent Nos. 5,124,314 and 5,175,145. Excess amylin action
mimics key features of Type II diabetes and amylin block-
ade has been proposed as a novel therapeutic strategy.
5   United States Patent No. 5,266,561, issued November 30,
1993, and United States Patent No. 5,281,581, issued
January 25, 1994, disclose the treatment of Type II
diabetes and insulin resistance with amylin antagonists.

It has previously been reported that infusion of
10 the amylin agonist, ^{25,28,29}Pro-h-amylin, decreased post-
prandial glucose concentrations in Type I diabetics.
Moyses & Kolterman, Drugs of the Future (May 1995). In a
single blind crossover study in 6 male subjects with Type
I diabetes, a 2-hour infusion of the amylin agonist,
15 ^{25,28,29}Pro-h-amylin at a rate of 150 μg/hr was reported to
significantly decrease post-prandial hyperglycemia follow-
ing ingestion of a mixed meal. This effect was confirmed
in a second study which employed an infusion rate of 50
μg/hr for 5 hours in 9 subjects with Type I diabetes. A
20 nutrient challenge was given either orally (in the form of
a Sustacal® meal) or as an intravenous glucose load of 300
mg/kg. Administration of ^{25,28,29}Pro-h-amylin reportedly led
to a significant decrease in post-prandial glucose excur-
sion after the oral Sustacal® meal, but not after the
25 intravenous administration of glucose, consistent with an
effect to delay gastric emptying and thus the gastrointes-
tinal absorption of nutrients. In a further double-blind,
placebo-controlled study, self-injected ^{25,28,29}Pro-h-amylin,
taken before meals thrice daily, also led to reduced
30 glucose excursion after a test meal. The patients contin-
ued their usual insulin regime. In the third study, a
statistically significant effect was seen with a dose of
30 μg of the amylin agonist.

Summary of the Invention
35   We have discovered that, unexpectedly, non-
insulin-taking Type II diabetic patients may be treated by

the administration of an amylin agonist in order to lower
their blood glucose concentrations. We have also discov-
ered that treatment of non-insulin-taking Type II diabetic
patients with an amylin agonist is particularly beneficial
5   for those patients having an HbAlc value above normal.·

In one aspect, the present invention is directed
to a method for the treatment of a non-insulin-taking Type
II diabetic subject comprising administering a therapeuti-
cally effective amount of an amylin agonist. By "non-
10 insulin-taking Type II diabetic subject" is meant a
subject who has Type II diabetes mellitus, but whose
diabetes is currently being managed without the use of
insulin, for example, by any combination of diet, exer-
cise, modification of lifestyle, or use of oral hypoglyce-
15 mic agents, such as biguanides and sulfonylureas. Diagno-
sis of patients afflicted with Type II diabetes is well
within the ability and knowledge of one skilled in the
art. For example, individuals over the age of 35 who have
symptoms of polydipsia, polyuria, polyphagia (with or
20 without weight loss) coupled with elevated plasma glucose
concentrations and without a history of ketoacidosis are
generally considered within the diagnosis of Type II
diabetes mellitus.   The presence of obesity, a positive
family history for Type II diabetes and normal or elevated
25 fasting plasma insulin and c-peptide concentrations are
additional characteristics of most patients with Type II
diabetes mellitus. By "therapeutically effective amount"
is meant an amount, either in single or multiple doses,
which beneficially reduces plasma glucose concentrations
30 in a subject afflicted with Type II diabetes mellitus.

In a preferred embodiment, the present invention
is directed to a method for the treatment of a non-insu-
lin-taking Type II diabetic subject comprising administer-
ing a therapeutically effective amount of an amylin
35 agonist wherein the subject has a HbAlc value above the
upper end of the range of HbAlc values for normal sub-
jects. HbAlc represents a post-translational modification

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of hemoglobin which occurs by a non-enzymatic process whereby glucose becomes attached to hemoglobin A, and typically reflects the average plasma glucose concentration over the preceding 120 days, the average lifespan of

5   a red blood cell. The upper end of the normal range for HbAlc values, that is, the upper end of the range for normal non-diabetic subjects, is generally understood to be from about 6.0% to about 7.5%. In another preferred embodiment, the subject has an HbAlc value of at least

10   about 8%.

In another aspect, the present invention is directed to a method of lowering blood glucose in a non-insulin-taking Type II diabetic subject comprising administering to said subject a blood glucose-lowering amount

15   of an amylin agonist. A glucose-lowering amount of an amylin agonist will preferably range from between about 0.05 µg/kg/day to about 10 µg/kg/day, more preferably, between about 0.05 µg/kg/day to about 6 µg/kg/day, and even more preferably, between about 1.0 µg/kg/day to about

20   4 µg/kg/day. For example, an effective blood glucose lowering amount of [25,28,29]Pro-h-amylin (AC137) will generally vary from about 0.05 µg/kg/day to about 10 µg/kg/day, preferably 0.1 µg/kg/day to about 6.0 µg/kg/day. A dose of from about 1.0 µg/kg/day to about 4.0 µg/kg/day is

25   preferred. In a preferred embodiment, the subject has an HbAlc value above the upper end of the normal range of HbAlc values for normal subjects. In another preferred embodiments of the present invention, the subject has an HbAlc value of at least about 8%.

30   In preferred embodiments of the present invention, the amylin agonist is selected from group consisting of [25,28,29]Pro-h-amylin (AC137), s-calcitonin and h-amylin. Particularly preferred is [25,28,29]Pro-h-amylin (AC137).

35   Administration of amylin agonists may be by various routes, including subcutaneously, intramuscularly, nasally or transdermally.

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<u>Brief Description of the Drawings</u>

The invention will be further described with
reference to the accompanying drawings in which:

Fig. 1 shows the correlation between AC137-
5 induced reduction in post-prandial hyperglycemia and HbAlc
status in insulin-treated and non-insulin-treated patients.. Patients are rank-ordered for HbAlc levels. The
correlation (r^2) was 0.56 in non-insulin-treated patients,
0.10 in insulin-treated patients, and 0.33 for all patients combined.
10 tients combined.

<u>Detailed Description of the Invention</u>

Amylin agonist agents may be identified by
activity in the receptor binding and soleus muscle assays
described below. Amylin agonist activity of compounds may
15 also be assessed by the ability to induce hypercalcemia
and/or hyperglycemia in mammals, or to reduce post-prandial plasma glucose levels, as described herein.

The nomenclature of various amylin agonist
compounds useful in the present invention can be used to
20 indicate both the peptide that the sequence is based on
and the modifications made to any basic peptide amylin
sequence, such as human amylin. An amino acid preceded by
a superscript number indicates that the named amino acid
replaces the amino acid normally present at the amino acid
25 position of the superscript in the basic amino acid
sequence. For example, "^{18}Arg^{25,28}Pro-h-amylin" refers to a
peptide based on the sequence of "h-amylin" or "human-
amylin" having the following substitutions: Arg replacing
His at residue 18, Pro replacing Ala at residue 25 and Pro
30 replacing Ser at residue 28. The term "des-^{1}Lys-h-amylin"
refers to a peptide based on the sequence of human amylin,
with the first, or N-terminal, amino acid deleted.

Preferred amylin agonist compounds des-^{1}Lys-h-
amylin, ^{28}Pro-h-amylin, ^{25,28,29}Pro-h-amylin, ^{18}Arg^{25,28}Pro-h-
35 amylin, and des-^{1}Lys^{18}Arg^{25,28}Pro-h-amylin, all show amylin
activity <u>in vivo</u> in treated test animals, provoking marked

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hyperlactemia followed by hyperglycemia. In addition to having activities characteristic of amylin, certain preferred compounds have also been found to possess more desirable solubility and stability characteristics when compared to human amylin. These preferred compounds include $^{25}Pro^{26}Val^{28,29}Pro$-h-amylin, $^{25,28,29}Pro$-h-amylin (also referred to herein as "AC-0137"), and $^{18}Arg^{25,28}Pro$-h-amylin.

The methods of the present invention employ an amylin agonists, including amylin or an amylin agonist analogue, for example, amylin receptor agonist analogues such as $^{18}Arg^{25,28}Pro$-h-amylin, des-$^{1}Lys^{18}Arg^{25,28}Pro$-h-amylin, $^{18}Arg^{25-28,29}Pro$-h-amylin, des-$^{1}Lys^{18}Arg^{25,28,29}Pro$-h-amylin, $^{25,28-29}Pro$-h-amylin, des-$^{1}Lys^{25,28,29}Pro$-h-amylin, and $^{25}Pro^{26}Val^{25,28}$-Pro-h-amylin. Examples of other suitable amylin agonist analogues include:

$^{23}Leu^{25}Pro^{26}Val^{28,29}Pro$-h-amylin;
$^{23}Leu^{25}Pro^{26}Val^{28}Pro$-h-amylin;
des-$^{1}Lys^{23}Leu^{25}Pro^{26}Val^{28}Pro$-h-amylin;
$^{18}Arg^{23}Leu^{25}Pro^{26}Val^{28}Pro$-h-amylin;
$^{18}Arg^{23}Leu^{25,28,29}Pro$-h-amylin;
$^{18}Arg^{23}Leu^{25,28}Pro$-h-amylin;
$^{17}Ile^{23}Leu^{25,28,29}Pro$-h-amylin;
$^{17}Ile^{25,28,29}Pro$-h-amylin;
des-$^{1}Lys^{17}Ile^{23}Leu^{25,28,29}Pro$-h-amylin;
$^{17}Ile^{18}Arg^{23}Leu$-h-amylin;
$^{17}Ile^{18}Arg^{23}Leu^{26}Val^{29}Pro$-h-amylin;
$^{17}Ile^{18}Arg^{23}Leu^{25}Pro^{26}Val^{28,29}Pro$-h-amylin;
$^{13}Thr^{21}His^{23}Leu^{26}Ala^{28}Leu^{29}Pro^{31}Asp$-h-amylin;
$^{13}Thr^{21}His^{23}Leu^{26}Ala^{29}Pro^{31}Asp$-h-amylin;
des-$^{1}Lys^{13}Thr^{21}His^{23}Leu^{26}Ala^{28}Pro^{31}Asp$-h-amylin;
$^{13}Thr^{18}Arg^{21}His^{23}Leu^{26}Ala^{29}Pro^{31}Asp$-h-amylin;
$^{13}Thr^{18}Arg^{}His^{23}Leu^{28,29}Pro^{31}Asp$-h-amylin; and,
$^{13}Thr^{18}Arg^{}His^{23}Leu^{25}Pro^{26}Ala^{28,29}Pro^{31}Asp$-h-amylin.

Still further amylin agonists including amylin agonist analogues are disclosed, and methods for making and using amylin agonists are further specified, in commonly owned in U.S. Patent Application, entitled "Novel

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Amylin Agonist Peptides and Uses Therfor" filed May 30,
1995 (docket no. 213/080) and corresponding PCT applica-
tion Publication No. WO 93/10146, published May 27, 1993,
the disclosures of which are hereby incorporated by this
5   reference. These agonists are useful in reducing blood
glucose concentrations in a non-insulin-using Type II
diabetic subject.

       The activity of amylin agonists may be evaluated
using certain biological assays described herein. The
10   receptor binding assay can identify both candidate amylin
agonists and antagonists and can be used to evaluate
binding, while the soleus muscle assay can be used to
distinguish between amylin agonists and antagonists.
Preferably, agonist compounds exhibit activity in the
15   receptor binding assay on the order of less than about 1
to 5 nM, preferably less than about 1 nM and more prefera-
bly less than about 50 pM. In the soleus muscle assay
these compounds preferably show EC_{50} values on the order of
less than about 1 to 10 micromolar.
20   The receptor binding assay is described in United
States Patent No. 5,264,372, issued November 23, 1993, the
disclosure of which is incorporated herein by reference.
The receptor binding assay is a competition assay which
measures the ability of compounds to bind specifically to
25   membrane-bound amylin receptors. A preferred source of
the membrane preparations used in the assay is the basal
forebrain which comprises membranes from the nucleus
accumbens and surrounding regions. Compounds being
assayed compete for binding to these receptor preparations
30   with [125]I Bolton Hunter rat amylin. Competition curves,
wherein the amount bound (B) is plotted as a function of
the log of the concentration of ligand are analyzed by
computer, using analyses by nonlinear regression to a
4-parameter logistic equation (Inplot program; GraphPAD
35   Software, San Diego, California) or the ALLFIT program of
DeLean et. al. (ALLFIT, Version 2.7 (NIH, Bethesda, MD
20892)). Munson, P. and Rodbard, D., Anal. Biochem.

107:220-239 (1980).

Assays of biological activity of amylin agonists, including amylin agonist analogue preparations, in the soleus muscle are performed using previously described

5   methods (Leighton, B. and Cooper, G.J.S., *Nature*, 335:632-635 (1988); Cooper, G.J.S., *et al.* *Proc. Natl. Acad. Sci. USA* 85:7763-7766 (1988)). In summary, amylin agonist activity is assessed by measuring the inhibition of insulin-stimulated glycogen synthesis in soleus muscle.

10 Amylin antagonist activity is assessed by measuring the resumption of insulin-stimulated glycogen synthesis in the presence of 100 nM rat amylin and an amylin antagonist. Concentrations of peptide dissolved in carrier-free buffers are determined by quantitative amino acid analy-

15 sis, as described therein. The ability of compounds to act as agonists in this assay is determined by measuring EC_{50} values. Standard errors are determined by fitting of sigmoidal dose response curves using a four parameter logistic equation (De Lean, A., Munson, P.J., Guardabasso,

20 V. and Rodbard, D. (1988) *ALLFIT*, Version 2.7, National Institute of Child Health and Human Development, N.I.H. Bethesda, MD, 1 diskette). A number of amylin agonists have been characterized using these biological assays. The compounds $^{18}Arg^{25,28}Pro$-h-amylin, des$^{1}Lys^{18}Arg^{25,28}Pro$-h-

25 amylin, $^{18}Arg^{25,28,29}Pro$-h-amylin, des-$^{1}Lys^{18}Arg$ $^{25,28,29}Pro$-h-amylin, $^{25,28,29}Pro$-h-amylin, des-$^{1}Lys^{25,28,29}Pro$-h-amylin, and $^{25}Pro^{26}Val^{25,28}Pro$-h-amylin were all found to compete with amylin in the receptor binding assay. These compounds have negligible antagonist activity as measured by the

30 soleus muscle assay and were shown to act as amylin agonists.

Effects of amylins or amylin agonists on gastric motility can be identified, evaluated, or screened for using the methods described in U.S. Applications Serial

35 No. 08/118,381, filed September 7, 1993, and U.S. Application Serial No. 08/302,069, filed September 7, 1994 (corresponding to PCT Application, Publication No. WO

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95/07098), the disclosures of which are hereby incorporat-
ed by reference, or other art-known or equivalent methods
for determining gastric motility. One such method for use
in identifying or evaluating the ability of a compound to
5   slow gastric motility, comprises: (a) bringing together a
test sample and a test system, said test sample comprising
one or more test compounds, and said test system compris-
ing a system for evaluating gastric motility, said system
being characterized in that it exhibits, for example,
10   elevated plasma glucose in response to the introduction to
said system of glucose or a meal; and, (b) determining the
presence or amount of a rise in plasma glucose in said
system. Positive and/or negative controls may be used as
well. Optionally, a predetermined amount of amylin
15   antagonist (e.g., $^{8-32}$salmon calcitonin) may be added to the
test system.

Amylin agonists such as those described above are
prepared using standard solid-phase peptide synthesis
techniques and preferably an automated or semiautomated
20   peptide synthesizer. Typically, an α-N-carbamoyl protect-
ed amino acid and an amino acid attached to the growing
peptide chain on a resin are coupled at room temperature
in an inert solvent such as dimethylformamide, N-methylpy-
rrolidinone or methylene chloride in the presence of
25   coupling agents such as dicyclohexylcarbodiimide and 1-
hydroxybenzotriazole in the presence of a base such as
diisopropylethylamine. The α-N-carbamoyl protecting group
is removed from the resulting peptide-resin using a
reagent such as trifluoroacetic acid or piperidine, and
30   the coupling reaction repeated with the next desired N-
protected amino acid to be added to the peptide chain.
Suitable N-protecting groups are well known in the art,
with t-butyloxycarbonyl (tBoc) and fluorenylmethoxycarbon-
yl (Fmoc) being preferred herein.
35   The solvents, amino acid derivatives and
4-methylbenzhydryl-amine resin used in the peptide synthe-
sizer are purchased from Applied Biosystems Inc. (Foster

City, CA), unless otherwise indicated. The side-chain
protected amino acids are purchased from Applied Biosyste-
ms, Inc. and include the following: Boc-Arg(Mts), Fmoc-
Arg(Pmc), Boc-Thr(Bzl), Fmoc-Thr(t-Bu), Boc-Ser(Bzl),

5   Fmoc-Ser(t-Bu), Boc-Tyr(BrZ), Fmoc-Tyr(t-Bu), Boc-Lys(Cl-
Z), Fmoc-Lys(Boc), Boc-Glu(Bzl), Fmoc-Glu(t-Bu), Fmoc-
His(Trt), Fmoc-Asn(Trt), and Fmoc-Gln(Trt). Boc-His(BOM)
is purchased from Applied Biosystems, Inc. or Bachem Inc.
(Torrance, CA). Anisole, methylsulfide, phenol, ethanedi-

10   thiol, and thioanisole are obtained from Aldrich Chemical
Company (Milwaukee, WI). Air Products and Chemicals
(Allentown, PA) supplies HF. Ethyl ether, acetic acid and
methanol are purchased from Fisher Scientific (Pittsburgh,
PA).

15   Solid phase peptide synthesis is carried out with
an automatic peptide synthesizer (Model 430A, Applied
Biosystems Inc., Foster City, CA) using the NMP/HOBt
(Option 1) system and Tboc or Fmoc chemistry (see, Applied
Biosystems User's Manual for the ABI 430A Peptide Synthe-

20   sizer, Version 1.3B July 1, 1988, section 6, pp. 49-70,
Applied Biosystems, Inc., Foster City, CA) with capping.
Boc-peptide-resins are cleaved with HF (-5°C to 0°C, 1
hour). The peptide is extracted from the resin with
alternating water and acetic acid, and the filtrates were

25   lyophilized. The Fmoc-peptide resins are cleaved accord-
ing to standard methods (Introduction to Cleavage Tech-
niques, Applied Biosystems, Inc., 1990, pp. 6-12). Some
peptides are also assembled using an Advanced Chem Tech
Synthesizer (Model MPS 350, Louisville, Kentucky).

30   Peptides are purified by RP-HPLC (preparative and analyti-
cal) using a Waters Delta Prep 3000 system. A C4, C8 or
C18 preparative column (10 μ, 2.2 x 25 cm; Vydac, Hesperi-
a, CA) is used to isolate peptides, and purity is deter-
mined using a C4, C8 or C18 analytical column (5 μ, 0.46

35   x 25 cm; Vydac). Solvents (A=0.1% TFA/water and B=0.1%
TFA/CH$_3$CN) are delivered to the analytical column at a
flowrate of 1.0 ml/min and to the preparative column at 15

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ml/min. Amino acid analyses are performed on the Waters
Pico Tag system and processed using the Maxima program.
The peptides are hydrolyzed by vapor-phase acid hydrolysis
(115°C, 20-24 h).   Hydrolysates are derivatized and
5 analyzed by standard methods (Cohen, S.A., Meys, M., and
Tarrin, T.L. (1989), The Pico Tag Method: A Manual of
Advanced Techniques for Amino Acid Analysis, pp. 11-52,
Millipore Corporation, Milford, MA).   Fast atom bombard-
ment analysis is carried out by M-Scan, Incorporated (West
10 Chester, PA). Mass calibration is performed using cesium
iodide or cesium iodide/glycerol.   Plasma desorption
ionization analysis using time of flight detection is
carried out on an Applied Biosystems Bio-Ion 20 mass
spectrometer.

15   Peptide compounds useful in the claimed methods
also be prepared using recombinant DNA techniques, using
methods now known in the art. See, e.g., Sambrook et al.,
Molecular Cloning: A Laboratory Manual, 2d Ed., Cold
Spring Harbor (1989).

20   The compounds referenced above form salts with
various inorganic and organic acids and bases. Such salts
include salts prepared with organic and inorganic acids,
for example, Hcl, HBr, H_2SO_4, H_3PO_4, trifluoroacetic acid,
acetic acid, formic acid, methanesulfonic acid, toluenesu-
25 lfonic acid, maleic acid, fumaric acid and camphorsulfonic
acid. Salts prepared with bases include ammonium salts,
alkali metal salts, e.g. sodium and potassium salts, and
alkali earth salts, e.g. calcium and magnesium salts.
Acetate, hydrochloride, and trifluoroacetate salts are
30 preferred. The salts may be formed by conventional means,
as by reacting the free acid or base forms of the product
with one or more equivalents of the appropriate base or
acid in a solvent or medium in which the salt is insolu-
ble, or in a solvent such as water which is then removed
35 in vacuo or by freeze-drying or by exchanging the ions of
an existing salt for another ion on a suitable ion ex-
change resin.

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Compositions useful in the invention may conveniently be provided in the form of formulations suitable for parenteral (including, intramuscular and subcutaneous) or nasal or transdermal, and/or suitably encapsulated or

5   otherwise prepared by another known methods for oral administration. A suitable administration format may best be determined by a medical practitioner for each patient individually. Suitable pharmaceutically acceptable carriers and their formulation are described in standard

10   formulation treatises, e.g., Remington's Pharmaceutical Sciences by E.W. Martin. See also Wang, Y.J. and Hanson, M.A. "Parenteral Formulations of Proteins and Peptides: Stability and Stabilizers," Journal of Parenteral Science and Technology, Technical Report No. 10, Supp. 42:2S

15   (1988).

Compounds useful in the invention can be provided as parenteral compositions for injection or infusion. Preferably, they are dissolved in an aqueous carrier, for example, in an isotonic buffer solution at a pH of about

20   4.3 to 7.4. These compositions may be sterilized by conventional sterilization techniques, or may be sterile filtered. The compositions may contain pharmaceutically acceptable auxiliary substances as required to stabilize the formulation, such as pH buffering agents. Useful

25   buffers include for example, sodium acetate/acetic acid buffers. A form of repository or "depot" slow release preparation may be used so that therapeutically effective amounts of the preparation are delivered into the bloodstream over many hours or days following transdermal

30   injection or delivery.

The desired isotonicity may be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol),

35   or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions. If desired, solutions of the above compositions may be

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thickened with a thickening agent such as methyl cellu-
lose.

Compositions useful in the invention are prepared
by mixing the ingredients following generally accepted
5   procedures. For example, the selected components may be
mixed in a blender or other standard device to produce a
concentrated mixture which may then be adjusted to the
final concentration and viscosity by the addition of water
or thickening agent and possibly a buffer to control pH or
10 an additional solute to control tonicity.

For use by the physician, the compositions will
be provided in dosage unit form containing an amount of an
amylin agonist, which will be effective in one or multiple
doses to control blood glucose at the selected level.
15 Therapeutically effective amounts of an amylin agonist are
those that decrease post-prandial blood glucose levels,
preferably to no lower than about 5 to 6 mM or such that
blood glucose levels are reduced as desired. In diabetic
or glucose intolerant individuals, plasma glucose levels
20 are higher than in normal individuals. In such individu-
als, beneficial reduction or "smoothing" of post-prandial
blood glucose levels, may be obtained. As will be recog-
nized by those in the field, in determining an effective
dose for a particular subject, a number of factors are
25 considered, including without limitation the patient's
size, age, and general health, the degree of or involve-
ment or the severity of the disease, the response of the
individual patient, the particular compound administered,
the mode of administration, the bioavailability character-
30 istics of the preparation administered, the dose regimen
selected and the use of concomitant medication.

The effective daily dose of amylin agonists,
including ^{18}Arg^{25,28}Pro-h-amylin, des-^{1}Lys^{18}Arg^{25,28}Pro-h-
amylin, ^{18}Arg^{25,28,29}Pro-h-amylin, des-^{1}Lys^{18}Arg-^{25,28,29}Pro-h-
35 amylin, ^{25,28,29}Pro-h-amylin, des-^{1}Lys^{25,28,29}Pro-h-amylin, and
^{25}Pro^{26}Val^{25,28}Pro-h-amylin, will typically be in the range of
0.05 μg/kg/day to about 10 μg/kg/day, preferably about

0.05 µg/kg/day to about 6.0 µg/kg/day and more preferably about 1.0 µg/kg/day to about 4.0 µg/kg/day administered in a single or divided doses. The exact dose to be administered is determined by the attending clinician and is
5   dependent upon where the particular compound lies within the above quoted range, as well as upon the age, weight and condition of the individual. Administration should begin at the first sign of symptoms or shortly after diagnosis of diabetes mellitus. Administration may be by
10 injection, preferably subcutaneous or intramuscular. Administration may also be nasally or transdermally. Orally active compounds may be taken orally, however dosages should be adjusted based on their potencies and bioavailabilities, as appropriate.
15   Peptide amylin agonists, for example, ^{25,28,29}Pro-h-amylin, may be administered in single or multiple doses, for example, two (BID), three (TID), and/or four (QID) times per day. BID doses preferably range from about 30 µg BID to about 150 µg BID, more
20 preferably from about 50 µg BID to about 60 µg BID. TID doses also preferably range from about 30 µg TID to about 150 µg TID, more preferably about 60 µg TID. QID doses preferably range from about 30 µg QID to about 60 µg QID, and more preferably about 30 µg QID. Preferably these
25 doses, which have been demonstrated to be effective in various human clinical trials, are administered subcutaneously.
        The following Example is illustrative, but not limiting of the methods of the present invention. Other
30 suitable amyli· agonist compounds that may be modified or adapted for use in the claimed methods are also appropriate and are within the spirit and scope of the invention.

Example

        A single-blind, placebo-controlled randomized,
35 two-period crossover clinical trial was performed to

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assess the effects of microinfusion of the amylin agonist, [25,28,29]Pro-h-amylin (AC137) on patients with Type II diabetes mellitus following a standardized oral test meal.

5   Patients who satisfied the screening requirements were randomized and participated in an investigation period which consisted of an acclimatization day (Study Day 1) followed by two treatment days (Study Days 2 and 3). Fourteen male and 10 female patients were enrolled in the trial Twelve patients were randomized to place-
10   bo/AC137 and 12 patients were randomized to AC137/placebo. Each study drug group had 6 insulin-treated patients and 6 non-insulin-treated patients. Of the 12 insulin-treated patients, 10 received insulin alone and 2 received an oral hypoglycemic agent. Of the 12 non-insulin-treated
15   patients, 7 were treated with an oral hypoglycemic agent. Thus, four groups of six patients were generated. Patients in Groups 1 and 2 received insulin. Patients in Groups 3 and 4 did not receive insulin. Patients in Groups 1 and 3 were treated with a 5-hour infusion of 100
20   µg/h AC137 on Study Day 2 followed by a 15-hour washout period and a 5-hour infusion of placebo on Study Day 3. Patients in Groups 2 and 4 were treated with a 5-hour infusion of placebo on Study Day 2 followed by a 15-hour washout period and a 5-hour infusion of 100 µg/h AC137 on
25   Study Day 3. Both AC137 and placebo were administered with an intravenous microinfusion pump. All patients consumed a standardized Sustacal® test meal (7 kcal/kg) 60 minutes after the start of study drug infusion on both treatment days. The Sustacal® test meal is a commercially
30   available nutritional supplement which contains 1.01 kcal/ml with the caloric composition being 24% protein, 21% fat and 55% carbohydrate. Sodium and calcium caseinates along with soy protein provide the protein calories; partially hydrogenated soybean oil provides the fat
35   calories; and sucrose and corn syrup provide the carbohydrate calories.

Each patient's usual insulin and/or oral hypogly-

cemic agent and caloric intake, as documented during the
screening visit, were stabilized during the 24-hour
acclimatization period (Study Day 1). Patients were
served breakfast at 8:00 a.m., lunch at 12:00 noon, and
5 dinner at 6:00 p.m. The patient's usual dose of insulin
and/or hypoglycemic agent was taken 30 minutes prior to
meals. An evening snack was served based on the usual
diet of the patients. Water was given as needed. All
patients followed this insulin/oral hypoglycemic and
10 dietary regimen throughout the investigation period.

Mean plasma glucose levels from Hours 1 through
5 following a Sustacal® test meal are presented in Table
1.

---

15 **TABLE 1.**   Mean Plasma Glucose Levels From Hours 1
                 Through 5 Following a Sustacal® Test Meal

	Mean ± SEM (mg/dl)		
Subgroup	AC137	Placebo	p-Value[a]
Insulin-Treated Patients (N=12)	183.1 ± 13.4	243.0 ± 16.5	0.0035
Non-Insulin-Treated Patients (N=12)	169.8 ± 15.7	183.2 ± 19.9	NS

[a] P-value for two-period crossover ANOVA. NS = Not
significant.

The mean plasma glucose levels from Hours 1
30 through 5 were statistically significantly lower following
the Sustacal® test meal when insulin-treated patients
received AC137 compared to when insulin-treated patients
receiving placebo. There was a mean reduction in glucose
levels of 23% in AC137-infused insulin-treated patients.
35   The mean glucose levels were not statistically
significantly different in AC137-infused non-insulin-
treated patients compared to placebo-treated patients.

The zero-hour $AUC_{glucose(1-5)}$ (area under the plasma
glucose concentration curve, above or below the baseline

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plasma glucose concentration, between hours 1 and 5 of the
test period calculated employing the trapezoidal rule) and
zero-hour C_{max} for plasma glucose (maximum plasma glucose
concentration) in insulin-treated and non-insulin-treated
5   patients following a Sustacal® test meal are summarized in
Table 2.

TABLE 2.	Zero-Hour $AUC_{(1-5)}$ and Zero-Hour C_{max} Following a Sustacal® Test Meal: Plasma Glucose		
Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value[a]
Insulin-Treated Patients (N=12)			
$AUC_{(1-5)}$ (mg-h/dl)	51.2 ± 52.0	284.4 ± 52.3	0.0012
C_{max} (mg/dl)	42.0 ± 15.0	118.1 ± 17.1	0.0007
Non-Insulin-Treated Patients (N=12)			
$AUC_{(1-5)}$ (mg-h/dl)	53.3 ± 27.4	124.3 ± 28.3	NS
C_{max} (mg/dl)	40.1 ± 10.2	66.9 ± 7.7	0.038

[a] P-value for two-period crossover ANOVA. NS = Not
25   significant.

The $AUC_{(1-5)}$ and C_{max} for plasma glucose in insulin-
treated and non-insulin-treated patients following a
Sustacal® test meal are summarized in Table 3.

TABLE 3.	$AUC_{(1-5)}$ and C_{max} Following a Sustacal® Test Meal: Plasma Glucose		
Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value[a]
Insulin-Treated Patients (N=12)			
$AUC_{(1-5)}$ (mg-h/dl)	736.2 ± 54.1	984.7 ± 67.6	0.0031
C_{max} (mg/dl)	213.3 ± 14.9	293.2 ± 21.9	0.0017
Non-Insulin-Treated Patients (N=12)			
$AUC_{(1-5)}$ (mg-h/dl)	681.3 ± 62.8	738.3 ± 80.0	NS
C_{max} (mg/dl)	197.1 ± 17.1	220.4 ± 20.0	0.048

[a] P-value for two-period crossover ANOVA.   NS = Not significant.

Glucose zero-hour $AUC_{(1-5)}$, zero-hour C_{max}, $AUC_{(1-5)}$, and C_{max} were statistically significantly lower when insulin-treated patients received AC137 compared to when insulin-treated patients received placebo. The effect was most pronounced for the zero-hour parameters. There was a mean reduction of 93% in zero-hour $AUC_{(1-5)}$ and a mean reduction of 66% in zero-hour C_{max} when insulin-treated patients received AC137.

In non-insulin-treated patients, only glucose zero-hour C_{max} and C_{max} were statistically significantly lower with AC137 infusion, although the reduction in zero-hour $AUC_{(1-5)}$ appears to be clinically meaningful.

Results from insulin-treated patients and non-insulin-treated patients were compared (ANOVA). After placebo infusion, glucose zero-hour $AUC_{(1-5)}$ was 2.3-fold higher (p=0.013) in insulin-treated patients than in non-insulin-treated patients. Glucose zero-hour C_{max} was 1.8-fold higher (p=0.012) in insulin-treated placebo patients than in non-insulin-treated placebo patients. Glucose $AUC_{(1-5)}$ and C_{max} also were high in insulin-treated placebo patients (p=0.028 and 0.023, respectively). When results

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from insulin-treated and non-insulin-treated patients were
compared (ANOVA) after AC137 infusion, there was no
statistically significant difference for glucose zero-hour
$AUC_{(1-5)}$, zero-hour C_{max}, $AUC_{(1-5)}$, and C_{max}.

5   The patient selection criteria included Type II
diabetic patients with levels of glycosylated hemoglobin
(HbA_{1c}) up to 13%, and, a correlation between AC137-induced
reduction in postprandial hyperglycemia and entry HbA_{1c}
levels was determined. Reduction in postprandial hyper-
10   glycemia was calculated by subtracting glucose zero-hour
$AUC_{(1-5)}$ with AC137 infusion from placebo infusion zero-hour
$AUC_{(1-5)}$. The results are presented in Figure 1 with
patients rank-ordered for HbA_{1c} levels.

The reduction in postprandial hyperglycemia
15   appeared to increase with HbA_{1c} levels in non-insulin-
treated patients. The correlation (r^2) was 0.56 in this
subgroup of patients, 0.10 in insulin-treated patients,
and 0.33 for all patients combined.

In order to further evaluate the relationship of
20   the reduction in postprandial hyperglycemia to HbA_{1c}
levels, glucose zero-hour $AUC_{(1-5)}$, zero-hour C_{max}, $AUC_{(1-5)}$,
and C_{max} following a Sustacal® test meal were evaluated in
insulin-treated and non-insulin-treated patients with HbA_{1c}
levels $\geq$ 8% and < 8%.

25   For patients with HbA_{1c} levels $\geq$ 8%, the zero-hour
$AUC_{(1-5)}$ and zero-hour C_{max} for plasma glucose in insulin-
treated and non-insulin-treated patients following a
Sustacal® test meal are summarized in Table 4.

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TABLE 4.	Zero-Hour $AUC_{(1-5)}$ and Zero-Hour C_{max} Following a Sustacal® Test Meal: Plasma Glucose in Patients With HbA_{1c} Levels $\geq$ 8%		
Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value[a]
Insulin-Treated Patients (N=9)			
$AUC_{(1-5)}$ (mg-h/dl)	63.1 ± 69.4	314.8 ± 67.3	0.0080
C_{max} (mg/dl)	48.8 ± 19.4	127.1 ± 21.4	0.0055
Non-Insulin-Treated Patients (N=5)			
$AUC_{(1-5)}$ (mg-h/dl)	-7.9 ± 32.8	189.8 ± 41.2	0.0323
C_{max} (mg/dl)	20.6 ± 12.0	79.4 ± 11.2	0.0238

[a] P-value for two-period crossover ANOVA. NS = Not significant.

For patients with HbA_{1c} levels $\geq$ 8%, the $AUC_{(1-5)}$ and C_{max} for plasma glucose in insulin-treated and non-insulin-treated patients following a Sustacal® test meal are summarized in Table 5.

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TABLE 5. $AUC_{(1-5)}$ and C_{max} Following a Sustacal® Test Meal: Plasma Glucose in Patients With HbA_{1c} Levels $\geq$ 8%

Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value[a]
Insulin-Treated Patients (N=9)			
$AUC_{(1-5)}$ (mg-h/dl)	747.9 ± 68.6	987.7 ± 75.4	0.0337
C_{max} (mg/dl)	220.0 ± 18.6	295.3 ± 23.6	0.0153
Non-Insulin-Treated Patients (N=5)			
$AUC_{(1-5)}$ (mg-h/dl)	871.4 ± 49.8	1033.8 ± 34.3	0.0369
C_{max} (mg/dl)	240.4 ± 16.4	290.4 ± 11.4	0.0362

[a] P-value for two-period crossover ANOVA. NS = Not significant.

For both insulin-treated and non-insulin-treated patients with HbA_{1c} levels $\geq$ 8%, glucose zero-hour $AUC_{(1-5)}$, zero-hour C_{max}, $AUC_{(1-5)}$, and C_{max} were statistically significantly lower when patients received AC137 compared to when patients received placebo. The differences for non-insulin-treated patients with HbA_{1c} levels $\geq$ 8% were statistically significant and were greater than those for all non-insulin-treated patients regardless of HbA_{1c} level.

For patients with HbA_{1c} levels < 8%, the zero-hour $AUC_{(1-5)}$ and zero-hour C_{max} for plasma glucose in insulin-treated and non-insulin-treated patients following a Sustacal® test meal are summarized in Table 6.

TABLE 6.	Zero-Hour $AUC_{(1-5)}$ and Zero-Hour C_{max} Following a Sustacal® Test Meal: Plasma Glucose in Patients With HbA_{1c} Levels < 8%		
Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value[a]
Insulin-Treated Patients (N=3)			
$AUC_{(1-5)}$ (mg-h/dl)	15.5 ± 30.4	193.1 ± 14.4	0.0324
C_{max} (mg/dl)	21.7 ± 10.8	91.0 ± 20.6	NS
Non-Insulin-Treated Patients (N=7)			
$AUC_{(1-5)}$ (mg-h/dl)	96.9 ± 32.8	77.6 ± 29.0	NS
C_{max} (mg/dl)	54.0 ± 13.4	58.0 ± 9.8	NS

[a] Two-period crossover ANOVA. NS = Not significant.

For patients with HbA_{1c} levels < 8%, the $AUC_{(1-5)}$ and C_{max} for plasma glucose in insulin-treated and non-insulin-treated patients following a Sustacal® test meal are summarized in Table 7.

30

TABLE 7.	AUC$_{(1-5)}$ and C$_{max}$ Following a Sustacal® Test Meal: Plasma Glucose in Patients With HbA$_{1c}$ Levels < 8%		
Subgroup/ Parameter	AC137 (Mean ± SEM)	Placebo (Mean ± SEM)	p-Value*
Insulin-Treated Patients (N=3)			
AUC$_{(1-5)}$ (mg-h/dl)	700.8 ± 84.2	975.8 ± 179.7	NS
C$_{max}$ (mg/dl)	193.0 ± 22.1	286.7 ± 61.9	NS
Non-Insulin-Treated Patients (N=7)			
AUC$_{(1-5)}$ (mg-h/dl)	545.5 ± 61.9	527.3 ± 41.8	NS
C$_{max}$ (mg/dl)	166.1 ± 20.3	170.4 ± 13.9	NS

* Two-period crossover ANOVA. NS = Not significant.

For insulin-treated patients with HbA$_{1c}$ levels < 8%, only glucose zero-hour AUC$_{(1-5)}$ was statistically significantly lower when patients received AC137 compared to when patients received placebo, although the differences for glucose zero-hour C$_{max}$, AUC$_{(1-5)}$, and C$_{max}$ may be clinically beneficial. For all insulin-treated patients regardless of HbA$_{1c}$ level, glucose zero-hour AUC$_{(1-5)}$, zero-hour C$_{max}$, AUC$_{(1-5)}$, and C$_{max}$ were statistically significantly lower when patients received AC137 compared to when patients received placebo. For non-insulin-treated patients with HbA$_{1c}$ levels < 8%, glucose zero-hour AUC$_{(1-5)}$, zero-hour C$_{max}$, AUC$_{(1-5)}$, and C$_{max}$ when patients received AC137 compared to when patients received placebo were not significantly different.

Thus, administration of an amylin agonist beneficially decreases blood glucose concentrations in patients with Type II diabetes even when such patients are not taking insulin, that is, for example, when such patients have not experienced primary dietary failure, particularly patients with Type II diabetes who have above-normal HbA1c values.

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WHAT IS CLAIMED IS:

        1. A method for the treatment of a non-insulin-
taking Type II diabetic subject comprising administering
to said subject a therapeutically effective amount of an
5   amylin agonist.

        2. A method of lowering blood glucose in a non-
insulin-taking Type II diabetic subject comprising admin-
istering to said subject a blood-glucose-lowering amount
of an amylin agonist.

10   3. A method according to claim 1 wherein said
subject has a HbAlc value above about the upper end of the
of range of HbAlc values for normal subjects.

        4. A method according to claim 2 wherein said
subject has an HbAlc value above about the upper end of
15   the range of HbAlc values for normal subjects.

        5. A method according to claim 1 wherein said
subject has an HbAlc value of about 8% or above.

        6. A method according to claim 2 wherein said
subject has an HbAlc value of about 8% or above.

20   7. A method according to any of claims 1-6
wherein said amylin agonist is ^{25,28,29}Pro-h-amylin.

        8. A method according to any of claims 1-6
wherein said amylin agonist is s-calcitonin.

        9. A method according to any of claims 1-6
25   wherein said amylin agonist is h-amylin.

        10. A method according to claim 1 wherein said
amylin agonist is administered intramuscularly or subcuta-

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neously.

      11.   A method according to claim 2 wherein said amylin agonist is administered intramuscularly or subcutaneously.

5   12.   A method according to claim 7 wherein said amylin agonist is administered intramuscularly or subcutaneously.

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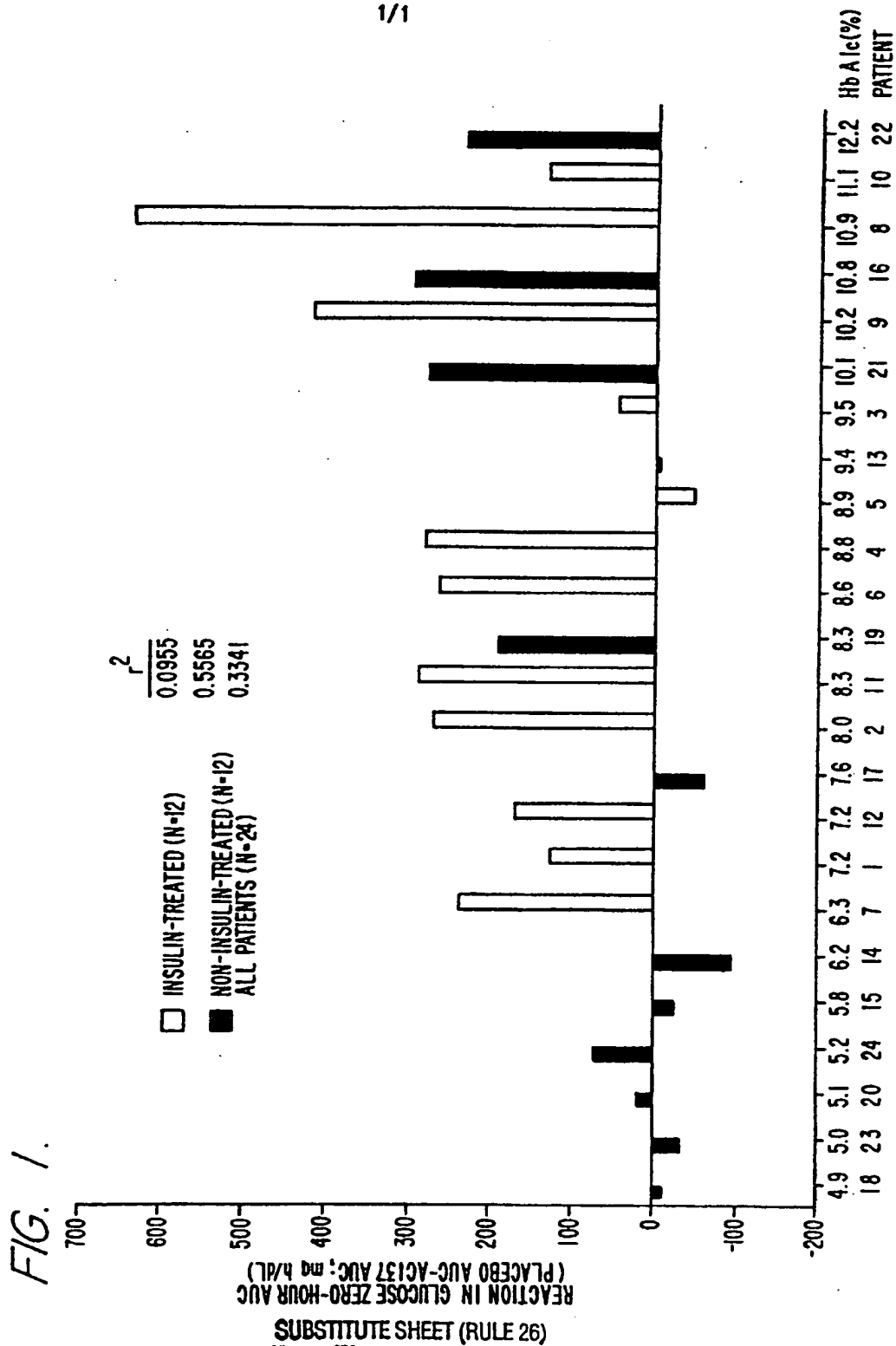
FIG. 1.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/23
US CL : 514/12, 21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,234,906 A (YOUNG ET AL) 10 August 1993, entire document.	1-12
X	WO 93/10146 A1 (AMYLIN PHARMACEUTICALS, INC.) 27 May 1993, entire document.	1-12

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	
"P"	document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 AUGUST 1996	27 SEP 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer PATRICK DELANEY
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